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(54) INTERLEUKIN-5 PRODUCTION INHIBITOR

(57) Object: to provide an erythromycin derivative having a potent interleukin-5 production inhibitory effect. Constitution: an interleukin-5 production inhibitor containing as the active ingredient an erythromycin derivative, such as 3-0-(4-biphenyl)acetyl-5-0-desosaminyl-6-0-methylerythronolide A 11,12-cyclic carbonate or 3-0-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-0-desosaminyl-6-0-methylerythronolide A, or a medicinally acceptable acid-addition salt thereof.

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Description

Technical Field

This invention relates to an inhibitor on interleukin 5 production which contains an erythromycin derivative as an active ingredient.

Background Art

Interleukin 5 (hereinafter abbreviated as IL-5) is known to be an important factor stimulating differentiation and growth of eosinophils which accelerate allergic inflammation. Therefore, an inhibitor on IL-5 production is useful for the treatment of various allergic diseases, such as bronchial asthma, allergic rhinitis, atopic dermatitis, drug allergy, and eosinophilic pneumonia.

Erythromycin is an antibiotic that has been of wide clinical use as a treating agent for infections caused by Gram-positive bacteria, some kinds of Gram-negative bacteria, Mycoplasma, etc. It has recently been reported that erythromycin and roxithromycin have an inhibitory activity on IL-5 production (Japanese Journal of Allergology, 44(3-2), p. 424 (1993)), but the IL-5 production inhibitory activity of erythromycin and roxithromycin is not sufficient.

An object of this invention is to provide an erythromycin derivative having a potent IL-5 production inhibitory activity.

Disclosure of Invention

The inventors of the present invention have extensively studied the IL-5 inhibitory activity of erythromycin derivatives, and as a result, have found that the following erythrimycin derivatives exhibit a potent IL-5 production inhibitory activity and thus completed the present invention.

The present invention provides an IL-5 production inhibitor comprising 3-O-(4-biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate, 3-O-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-O-desosaminyl-6-O-methylerythronolide A, or a pharmaceutically acceptable acid addition salt thereof as an active ingredient.

The pharmaceutically acceptable acid addition salt for use in the invention includes an acetate, a propionate, a butyrate, a formate, a trifluoroacetate, a maleate, a tartrate, a citrate, a stearate, a succinate, an ethylsuccinate, a lactobionate, a gluconate, a glucoheptonate, a benzoate, a methanesulfonate, an ethanesulfonate, a 2-hydroxyethanesulfonate, a benzenesulfonate, a p-toluenesulfonate, a laurylsulfate, a malate, an aspartate, a glutamate, an adipate, a cysteinate, a hydrochloride, a hydrobromide, a phosphate, a sulfate, a hydroiodide, a nicotinat , an oxalate, a picrate, a thiocyanate, an undecanoate, a salt of an acrylic acid polymer, and a salt of a carboxyvinyl polymer.

Th 3-O-(4-biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate used in the invention can be prepared according to, for example, the process described in WO 93/13116.

The 3-O-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-O-desosaminyl-6-O-methylerythronolide A used in the invention can be prepared by, for example, as follows. Step (1): 5-O-Desosaminyl-6-O-methylerythronolide A dissolved in an appropriate solvent is reacted with pentafluoronitrobenzene in the presence of a base. Suitable solvents include acetone, tetrahydrofuran, N,N-dimethylformamide, dimethyl sulfoxide, dioxane, and mixtures thereof. Suitable bases include sodium hydride, sodium hydroxide, and potassium hydroxide. The reaction temperature ranges from -20° to 50°C, preferably 0° to 25°C.

The compounds according to the invention can be administered orally or non-orally in the form of tablets, capsules, granules, dusts, powders, troaches, ointments, creams, emulsions, suspensions, suppositories, and injectable solutions. These dose forms can be prepared in a conventional manner, for example, the methods specified in Japanese Pharmacopoeia (12th Rev.). An appropriate dose form is chosen depending on the conditions and age of a patient and the purpose of treatment. In the preparation of various dose forms, commonly employed vehicles (e.g., crystallien cellulose, starch, lactose, mannitol), binders (e.g., hydroxypropyl cellulose, polyvinylpyrrolidone), lubricants (e.g., magnesium stearate, talc), disintegrants (e.g., craboxymethyl cellulose calcium), and the like can be used.

The dosage of the compound of the present invention ranges, for example, from 50 to 2000 mg in 2 to 3-divided doses per day in orall administration to adults, while appropriately varying depending on the age, body weight and conditions of a patient.

Industrial Applicability

The compounds according to the invention exhibit a potent IL-5 production inhibitory activity and are useful as an IL-5 production inhibitor in humans and animals (inclusive of livestock). Thus, the compounds of the invention are effective on diseases caused by IL-5 production, i.e., various allgergic diseases, such as bronchial asthma, allergic rhinitis, atopic dermatitis, drug allergy, and eosinophilic pneumonia.

Best Mode for Carrying out Invention

The present invention is now illustrated in greater detail with reference to Examples.

Example 1: Preparation of 3-O-(4-Biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-Cyclic Carbonate

(1) In 100 ml of aceton was dissolved 11.78 g (0.02 mol) of 5-O-desosaminyl-6-O-methylerythronolid A, and 2.27 ml (0.024 mol) of ac tic anhy-

dride was added to the solution under cooling with ice, followed by stirring at room temperature for 6 hours. Acetone was removed by evaporation under reduced pressure, and the residue was extracted with dichloromethane. The dichloromethane layer was washed successively with a saturated sodium hydrogencarbonate aqueous solution and a saturated sodium chloride aqueous solution, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to remove the solvent. The residue was recrystallized from ether/n-hexane to gave 12.17 g of 2'-O-acetyl-5-O-desosaminyl-6-O-methylerythronolide A as white powder.

Melting point: 158-160°C

Mass (FAB) m/z: 632 $[MH]^+$

^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm:

2.07 (3H, s), 2.26 (6H, s), 2.95 (3H, s), 3.26 (1H, s), 3.96 (1H, s)

IR (KBr), cm^{-1}: 3469, 1750, 1733, 1693

(2) In 500 ml of dichloromethane was dissolved 50 g (84.8 mmol) of the compound obtained in (1) above, and 102.6 ml (1.27 mol) of pyridine was added to the solution under ice-cooling. A solution of 25.4 ml (212 mmol) of trichloromethyl chloroformate in 40 ml of dichloromethane was added thereto dropwise at the same temperature, followed by stirring for 5.5 hours. Cold water and a saturated sodium hydrogencarbonate aqueous solution were added to the reaction mixture in small portions, and the mixture was extracted with dichloromethane. The dichloromethane layer was washed successively with a saturated sodium hydrogencarbonate aqueous solution and a saturated sodium chloride aqueous solution, and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (eluent: acetone/n-hexane/triethylamine=6 to 10:10:0.2) to give 41.93 g of 2'-O-acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate as a white foamy substance.

^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm:

2.05 (3H, s), 2.25 (6H, s), 2.92 (3H, s), 4.57 (1H, d, J=9Hz), 4.74 (1H, s), 4.75 (1H, dd, J=10Hz, 9Hz), 5.13 (1H, dd, J=12Hz, 2Hz)

(3) In 30 ml of dichloromethane was dissolved 1.274 g (6 mmol) of 4-biphenylacetic acid, and 0.84 ml (6 mmol) of triethylamine was added thereto. To the solution was added 0.75 ml (6 mmol) of pivaloyl chloride under cooling with ice, followed by stirring for 30 minutes. A solution of 1.65 ml (20.4 mmol) of pyridine and 1.31 g (2 mmol) of the compound obtained in (2) above in 10 ml of dichloromethane was added thereto. After stirring for 18 hours, the reaction mixture was post-treated in the same manner as in (1) above. The solvent was evaporated under reduced pressure, and the resultant crude product was purified by silica gel column chromatography (eluent: acetone/n-hexane/triethylamine =4:10:0.05) to give 580 mg of a pale yellow foamy substance. The resulting compound (580 mg, 0.72 mmol) was heated under reflux in 10 ml of methanol for 3 hours, and the solvent was removed by evaporation under reduced pressure to give 490 mg of the title compound as a pale yellow powder.

^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm:

2.21 (6H, s), 3.01 (3H, s), 3.90 (1H, d, J=7Hz), 4.76 (1H, s), 5.10 (1H, d, J=11Hz), 7.30-7.49 (5H, m), 7.55-7.61 (4H, m)

Example 2: Preparation of 3-O-(2-Nitro-3,4,5,6-tetrafluoro)-phenyl-5-O-desosaminyl-6-O-methylerythronolide A

To a solution of 1.178 g (2 mmol) of 5-O-desosaminyl-6-O-methylerythronolide A and 4.02 ml (10 mmol) of pentafluoronitrobenzene in 10 ml of tetrahydrofuran was added 240 mg (6 mmol) of 60% sodium hydride, followed by stirring at room temperature for 2 hours. The reaction mixture was extracted with ethyl acetate, and the extract was washed with a saturated sodium chloride aqueous solution and dried over anhyrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: chloroform/methanol/aqueous ammonia=19:1:0.1) to give 210 mg of the title compound.

Mass (FAB) m/z: 783 $[MH]^+$

^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm: 2.25 (6H, s), 3.05 (3H, s)

Example 3:

Ten grams of the compound prepared in Example 1, 550 g of lactose, 300 g of corn starch, 100 g of carboxymethyl cellulose calcium, and 30 g of polyvinylpyrrolidone were mixed well, granulated using ethanol, dried and classified in an usual manner. The granules were mixed with 10 g of magnesium stearate, and the mixture was tableted in a usual manner to obtain tablets each weighing 100 mg.

Example 4:

The action on IL-5 production in mice was examined according to the following method as disclosed in M. Hikida, et al., Immunology Letters, Vol. 34, pp. 297-302 (1992).

Murine Th2 clone (D10.G4.1 cells) was purchased from ATCC. Antigen presenting cells were prepared by suspending 1 x 10^7 spleen cells of a 8-week-old female C3H/HeN mice in 5 ml of an RPMI-1640 medium and incubated together with 50 μg/ml of mitomycin C (MMC) at 37°C for 30 minutes and then washed with three 50 ml portions of RPMI-1640. To RPMI-1640 containing 10% bovin serium were added 0.5 U of IL-2 (produced by Genzyme) and 5 x 10^{-5} M of 2-mercaptoethanol to prepare a medium for tissue culture. A test compound

was dissolved in dimethyl sulfoxide (DMSO) and diluted with the tissue culture medium to have a final DMSO concentration of 0.1% and a varied test compound concentration.

In each well of a 96-well microtiter plate (produced by Corning Glass Works) were put each 50 μl/well of 4 x 10^5 cells/ml of D10.G4.1 cells, 2 x 10^6 cells/ml of MMC-treated antigen presenting cells, 400 μg/ml of conalbumin (produced by Sigma) as an antigen and the solution of the test compound in the tissue culture medium (to make 200 μl/well), and incubated in an incubator under 5% CO_2 at 37°C for 48 hours. After completion of the incubation, the supernatant liquor of the culture was collected, and the cells were seprated by centrifugation. The IL-5 in the supernatant liquor was determined with an IL-5 ELISA kit produced by ENDOGEN. The inhibitory effect of the test compound on IL-5 production, expressed in terms of 50% inhibitory concentration (IC_{50}), is shown in Table 1 below.

TABLE 1

Test Compound	IC_{50} (M)
Compound of Example 1	<4 x 10^{-7}
Compound of Example 2	<1 x 10^{-7}
Roxithromycin	9.3 x 10^{-7}
Erythromycin	4.5 x 10^{-6}

Example 5:

The compounds of Examples 1 and 2 were orally administered to ICR male mice grouped in fives. No death was observed at a dose level of 100 mg/kg, proving that the compounds are of high safety.

Claims

1. An interleukin 5 production inhibitor comprising 3-O-(4-biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate, 3-O-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-O-desosaminyl-6-O-methylerythronolide A, or a pharmaceutically acceptable acid addition salt thereof as an active ingredient.

2. Use of 3-O-(4-biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate, 3-O-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-O-desosaminyl-6-O-methylerythronolide A, or a pharmaceutically acceptable acid addition salt thereof for the production of an interleukin 5 production inhibitor.

3. A method for treating an allergic disease comprising administering 3-O-(4-biphenyl)acetyl-5-O-desosaminyl-6-O-methylerythronolide A 11,12-cyclic carbonate, 3-O-(2-nitro-3,4,5,6-tetrafluoro)phenyl-5-O-desosaminyl-6-O-methylerythronolide A, or a pharmaceutically acceptable acid addition salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/01591

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 A61K31/70, C07H17/08
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 A61K31/70, C07H17/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAKESHITA, K. et al. "Immunological and anti-inflammatory effects of clarithromycin : inhibition of interleukin/production of murine peritoneal macrophages", Drugs Exp. Clin. Res., 15(11-12), (1989) p. 527-533	1 - 2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
October 24, 1995 (24. 10. 95)	November 14, 1995 (14. 11. 95)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/01591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 3
because they relate to subject matter not required to be searched by this Authority, namely:
The invention as set forth in claim 3 relates to methods for treatment of the human or animal body by therapy.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)